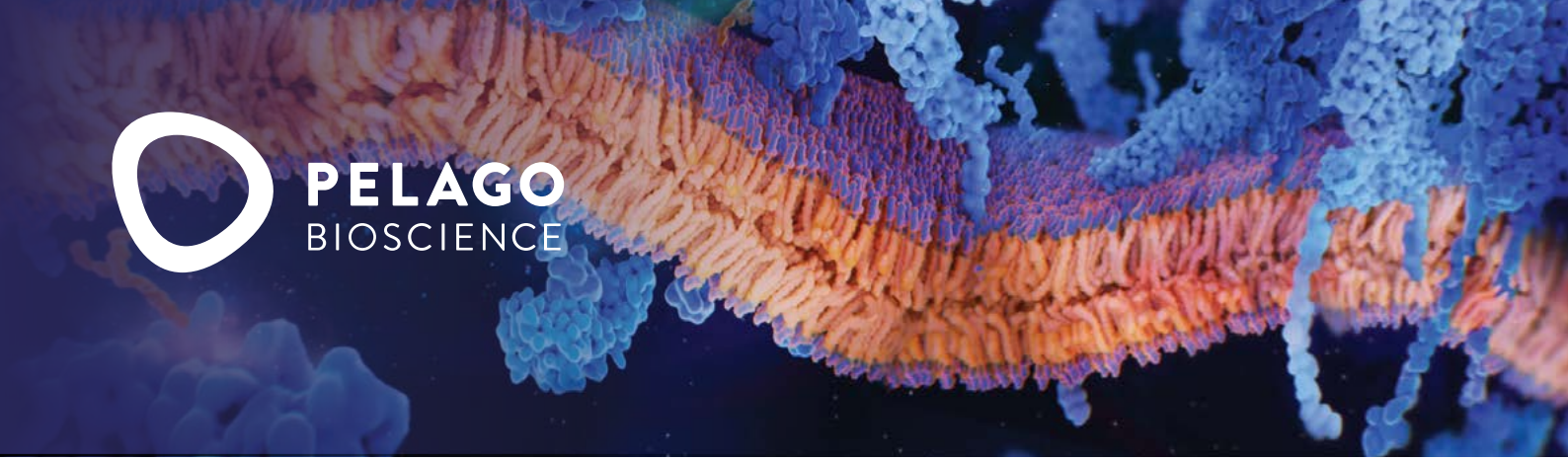

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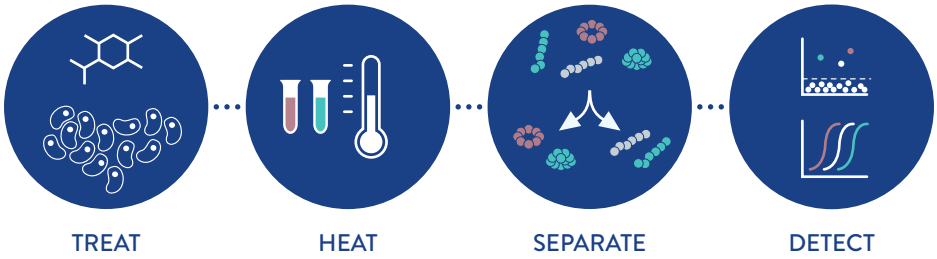
How does cellular target engagement relate to efficacy?

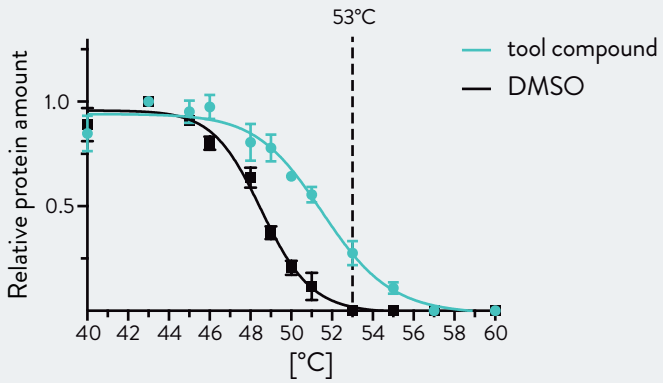
A drug's efficacy depends on how well it binds to its intended target and the resulting change in protein function, the relationship between target engagement and the ability to initiate a response. Lack of efficacy due to insufficient target engagement is a major contributor as to why drugs do not reach phase III clinical trials.

The patented Cellular Thermal Shift Assay (CETSA®), helps you to circumvent this persistent problem. CETSA® EC_{50} determines the potency of compounds directly in the cells or tissues of interest, providing crucial information about target engagement independent from function, early in the drug discovery pipeline.



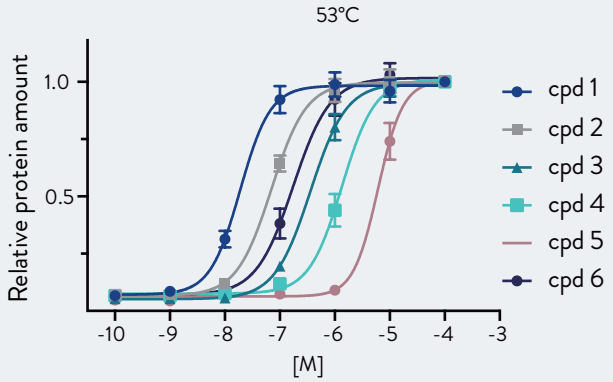
CETSA® can be used to verify and validate target engagement

STEP 1.



Step 1 – During assay development the CETSA® melt curves are generated by heating the compound treated sample and the vehicle control (DMSO) in ascending order. When the compound-induced shift is characterised, a fixed temperature is used to establish the concentration response curve (53°C in this example)[1,2].

STEP 2.



Step 2 – To determine the CETSA® EC_{50} potencies, cells are incubated with increasing compound concentration and heated to a fixed temperature (53°C in this experiment). This yields a concentration response relationship between target protein and compound concentration. In this example compound 1 exhibits the highest potency from the compounds tested, significantly more potent than compound 5.

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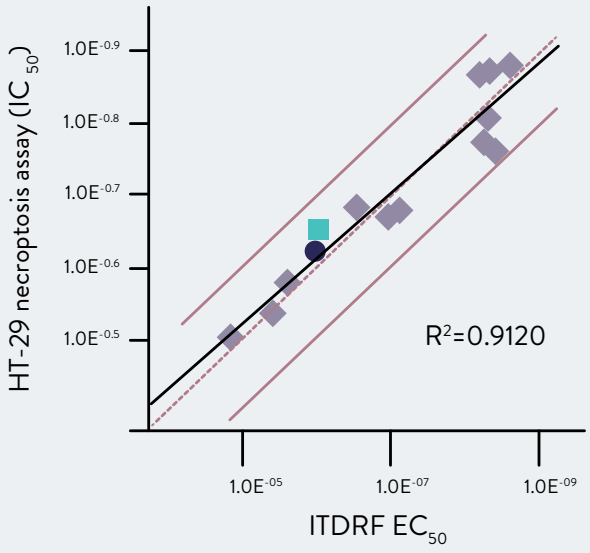
CETSA® EC_{50} provides valuable information on your compound's potency. Because of the relative measure it is advisable to test three or more compounds, at a fixed temperature. This will allow you to rank and prioritise your leads. The CETSA® EC_{50} values are dependent on the context of the cellular environment and assay parameters and in the proof of concept publication are called isothermal dose response fingerprints (ITDRFs)[1].

CETSA® EC_{50} correlates with other measures of affinity

Commonly used affinity assays usually measure functional activity for quantitative readouts. A battery of compounds can be compared and evaluated across multiple platforms including CETSA®.

Receptor Interacting Protein Kinase 1 (RIPK1) is implicated in a variety of human diseases such as acute and chronic inflammation, multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS)[3]. Takeda® developed a panel of RIPK1 inhibitors and subsequently evaluated 14 compounds using CETSA® target engagement within *in vitro* cell lines, *in vivo* dosed mice as well as mouse and human Peripheral Blood Mononuclear Cells (PBMCs). The results are compared with validated functional assays. In this example CETSA® EC_{50} values are correlated with a necroptosis assay, which is a cellular assay where cytotoxicity is measured upon treatment with necroptosis inducers in the presence or absence of inhibitors. The CETSA® EC_{50} values for the 14 compounds are correlated with the half maximal inhibitory concentration IC_{50} in the *in vitro* necroptosis assay.

CETSA® determines target engagement independent from function. The CETSA® EC_{50} values correlate well with the cellular inhibition of RIPK1. Both assays have a cellular context in which the inhibitors have to cross the plasma membrane prior to target engagement in order to alter the assay readout.



Correlation between functional cellular activity and CETSA: Scatterplot showing IC_{50} values of human HT-29 cells necroptosis inhibitory activities against EC_{50} values of half-maximal stabilisation of endogenous RIPK1 for 14 representative RIPK1 inhibitors.

CETSA® EC_{50} provides you with the relative potency of your compound, allowing you to rank and prioritise lead compounds in the native physiological environment. CETSA® can accelerate your drug discovery pipeline by evaluating your compound's target engagement in relevant tissues – before risks and costs escalate.

References

1. Martinez Molina et al. Science 2013
2. Jafari et al. Nature Protocols 2014
3. Ishii et al. Nature Scientific Reports 2017

Figures in this application note are modified from original.